

Figure 1: Isolation of monokaryotic strain deficient in laccase activity.

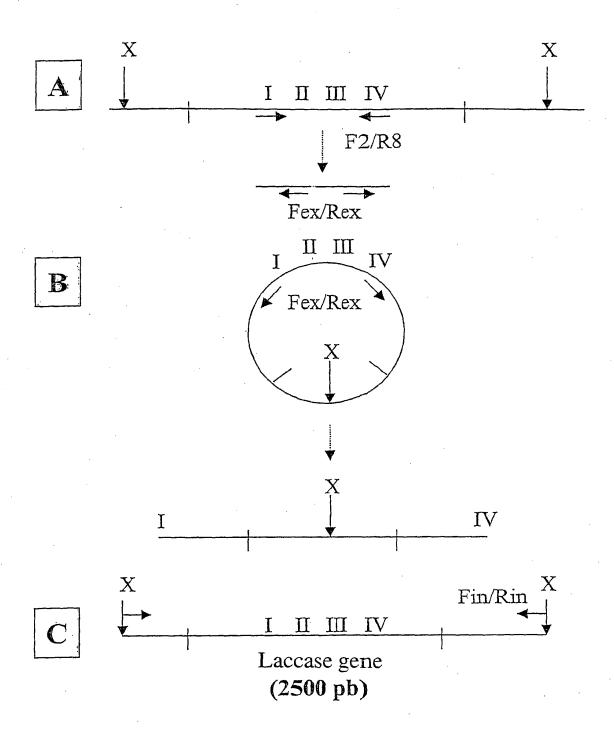


Figure 2: Isolation of the gene coding for the laccase of *Pycnoporus cinnabarinus* laccase.

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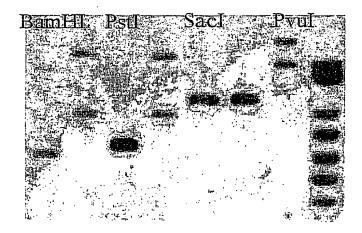


Figure 3 : Southern blot study of the gene coding for the laccase of *Pycnoporus cinnabarinus*.

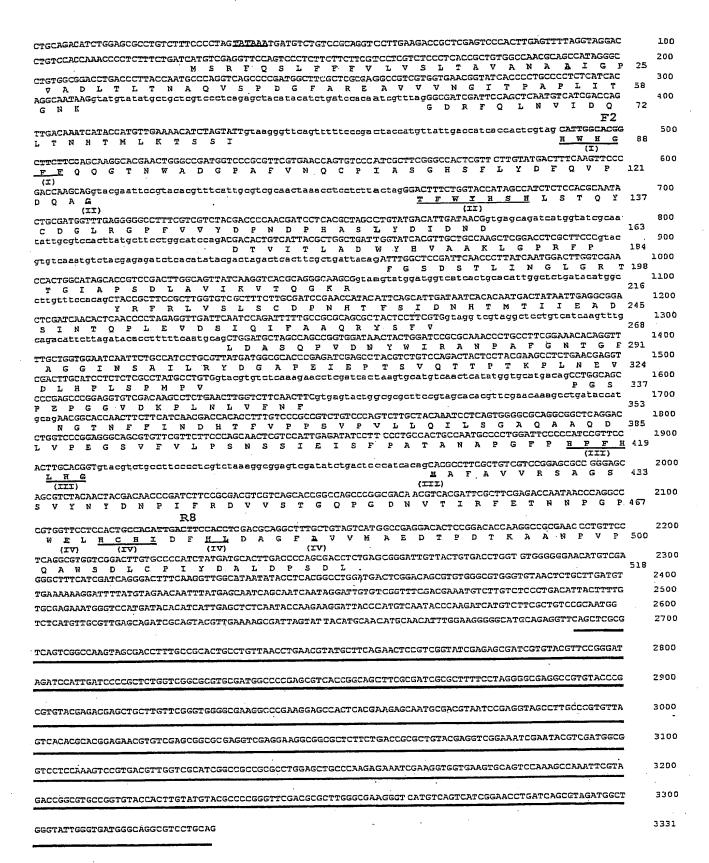


Figure 4: Sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus*

AGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCAGGGCAGCGACGTA CCGGCCGGCGTGCGCCATTGAGGTACATGAGCGGGGCGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCG CGGCGATGAAACGTTTCCCACCATTGGGAAGAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGC GGCGTCGCCCTTTGCCGCAGAGGCCGGCGGCGACATGCACAGCGAAGGTCCGTTGCGGATGGGAAGCAGG CAATCAGTGGGTGTCCTACGCCGCCACGATGGTCGGGGGAGCGTAGGCGCCCTCCCATAAGGCGGCAAGCATC ATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACC ATCGCCATCCTCAGCCTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGC TTCTTGGATGATCATGTCGTAACTTTTTCTGACCTCGTCGGTGGTACGCATGGCAGGATTGAGCATTACGGT ATGCCTCCCATTCATAAACGATAACCCCTTCCTTCAGGTTGGTCATCTCCATAGAGCGGCACGCTCTCAAGG CCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCATGGGATC ACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGT TCAGTCACCACATGGCAAAAAAGCTGCACCATACTCTTTATGGTGAGTTGTTCGTGAGTGGTATACAGTCAT TCATGAGGGAATGCCCACCGGATAGGGTĞTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGT CCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAAACAGCGTTGACTGTGAATCCCTGGTGTGT GTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTGCGAGCGGCACAG GCAGGCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGG AGCGAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGAT TGGACAAGGCCGAGCTATGATAGCTTGCTCCCGAAGTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGT CTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAATGCGCACACACGGAGGCTTTA CATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCT CTCATGACGTCCGCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATGGTAGTGGA GTCAGCCTGGCCAGTGCGTAGTCCCGTCTCTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTTGC TTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTGCACGCCCAGAGGTATTGGGCTCGTCA GATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCTATAGCCCTT CTTCGCGCGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTAC CGAACAATTGACTTACCGACATCCTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCC GCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGACGGCCCGCTCATCAGGACCAGACCAGTCTCAAT GTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTGGTGCACCGTCGCTTAC GATCATG

Figure 5: Sequence of the promoter sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus* (up to the ATG coding for the methionine of the laccase).

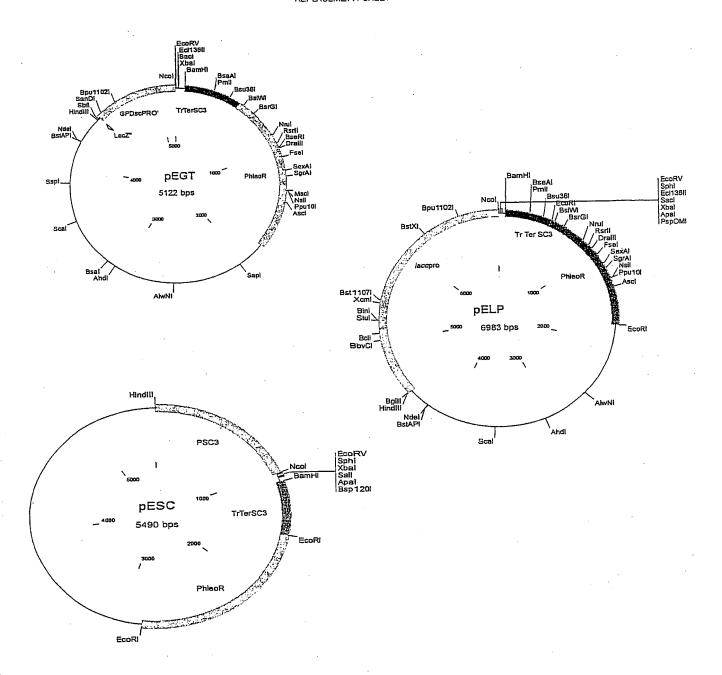


Figure 6: Restriction map of the three expression vectors used for the production of laccase in *Pycnoporus cinnabarinus*.

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CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCCCTTAAGACGCGTGGATCCGCAGGTGAAC GCGCCTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGT TTCTCTATCCGCTCAGTCACGCGGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACACCGCATGACGACATGGCGAACCTAAG GGGGGTACAAAAGGAGGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCT CAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATC ATTTTGGGATCGTACAATTATTAGACATGTTGTACAACGTTACATTCCTTTCTTCTTTTACTCTCCGGCCCAGTCTATGTAGAGGTAAA GTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACG AGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTTCACGAAGCTCTTGCTGATCGCGATGCCG GGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCGG CTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCAC CCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACCGGC CCGGAACGGCACTGGTCAACTTGGCCATGCATGGTGATGGGCATTATGTGTGATGGGATGCGATGGGAGAGGGAAGTGCTCTGGATG CCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGTCCTTCTCGCACC TGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAGGCTCTCGCGC CACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGGGAATTCGT AATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACATACGAGCCGGAAGCATAAAGTGTAAAG CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCT GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCG GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACG AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTC ACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGCACGAACCCCCGGTTCAGCCCGACCGCTGCGCC TTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCCGCCACTGGTAACAGGATTAGCAGA GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGTTT GCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAA TCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTC ATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGGGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG $\tt CGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCT$ AGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGŤACTCAACCAAGTCATTCTGAGAATAG TGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATT GGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGA TCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGAC ACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTG AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCA TGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGC AGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGG TGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACÁGATGCGTA AGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTA CGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGAC GCGGGCCCCGCCGCCCCCTCGGCCAGCGGTGTATCTACGAACGGAACTGGGAGGCGACTCGGAAGAGTTTGGTTAGAAAGGG GAACACCATCGCGGACGGCCCAGTGCTCTGGDCAGCTGAGCGTGCATTGTGTTCAATTCTGACCTGTGGCATGTAAGGAACGTGCTC GGGATCGGAGGGTGGCGCGAGAGCCTCTTCGGTGTGAGATTAGTAACTGTACTGCGAAGCCGCGGAGGGGTTAGGATGAGAGGTAG ACAGGGTCGCAGCCCAGGTGCGAGAAGGACTGCGAAGGACTGTTCTTCGACCGCGCACCTGCAATTGCGCGCATGGATAGAATAGA

Figure 7: Nucleotide sequence of the vector pEGT, containing the gpd gene promoter (4480-5112), a phleomycin resistance marker (507-1822) and the sc3 gene terminater (71-507).

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REPLACEMENT SHEET

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Figure 8: Nucleotide sequence of the vector pESC, containing the sc3 gene promoter (1-1033), a phleomycin resistance marker (1540-2855) and the sc3 gene terminater (1104-1540).

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REPLACEMENT SHEET

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCGCCTTAAGACGCGTGGATCCGCAGGTGAACGCGC CTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGT TCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGT GTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCTCAAAAAGGTTCTGCCCGTACG CCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATCATTTTGGGATCGTACAATTATTAGA CATGTTGTACAACGTTACATTCCTTTCTTTTACTCTCCGGCCCAGTCTATGTAGAGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTT AGAGCGCGCCGTCTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGGCGGGAGTAGCCCC ACCCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCAGTCCTGCTCCTCCGGCCACGAAGTGCACGCAGTTG GACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGG TGATGGGATGCGATGGGAGAGGGAAGTGCTCTGGATGGGAGTGCTGGAGAAAGAGGGGAGACGGCGGGGGGGCGCCCTTTTATACCCACG CCGTCGGGCGCCACCACCAGCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGA AGAACAGTCCTTCGCAGTCCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTA ATCTCACACCGAAGAGGCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTC ARATCAGATCCCCGGGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC AACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTG CAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCC CCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCT CACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTT ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAG TTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTA.AATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTA AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCG TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGG TATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAA GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACG GGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACA GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATT TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTA.GAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAG TGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGG TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCA GGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTG TGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGT GCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG ACGTTGTAAAACGACGGCCAGTGCCAAGCTTAGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCA TTGGGAAGAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGCGGCGTCGCGCCTTTGCCGCAGAGGCCGGCGGCGACATGCA

Figure 9: Nucleotide sequence of the vector pELP, containing the laccase gene (promoter 4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene terminater (71-507) (continuation of the sequence on the following page).

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Figure 9: Nucleotide sequence of the vector pELP (continuation), containing the laccase gene (promoter4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene ternminater (71-507).

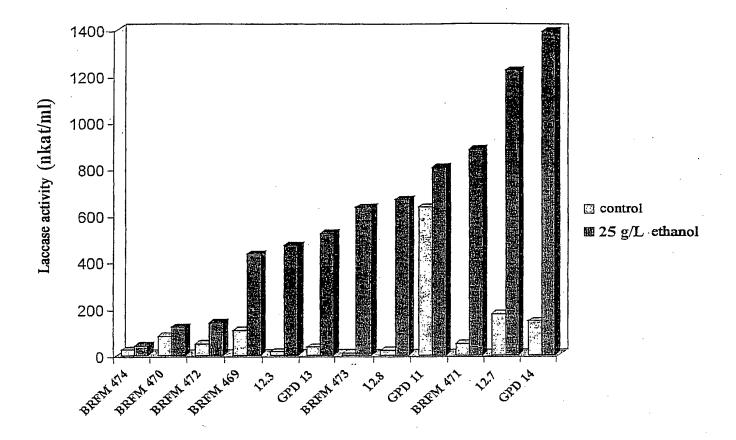
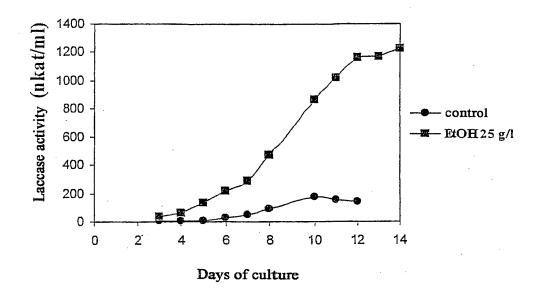


Figure 10: Results of production of the transformants having the most significant activities. The culture was carried out with or without (control) ethanol.



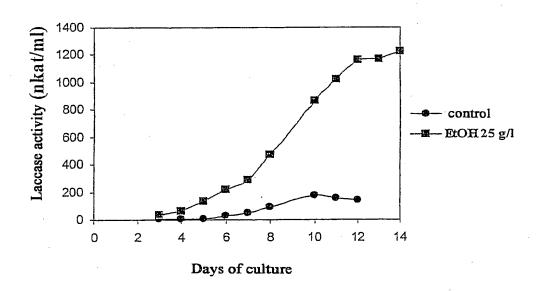


Figure 11: Monitoring of the laccase activities of the transformants GPD 14 and 12.7 as a function of time with or (control) without ethanol

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REPLACEMENT SHEET

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S V H A A V G P V T D L T L I V D T V A P D G A A F A R E
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600
800
900
                                     IAPGGDS
                                  C P
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                                              V E H G K
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Gene of the laccase of Halocyphina villosa